

REMARKS

Claims 1, 3, 4, 7-9, 11-20, 22, 24, 26 and 27 remain pending in this application.

Claims 1 and 27 have been amended to recite (i) options for substituents on ring A in accordance with the disclosure at page 28, lines 5-23, for example; (2) options for Y and Ya in accordance with the disclosure at page 29, lines 11-23, for example; and (3) that R¹, R², R⁴ and R⁵ can be C₁₋₆ alkyl in accordance with the disclosure at page 33, lines 12 and 30, respectively, for example. Claim 4 has been amended by deleting reference to R³, now defined in claim 1. Claim 7 has been amended to define Yd in the manner disclosed in the specification at page 40, lines 6-28 and page 37, lines 2-20. Finally, claims 19, 20, 24 and 26 have been amended to more clearly define the disease conditions to be treated in accordance with the disclosure at page 69, lines 15-20, for example, and to delete reference to "diabetic complications" and either "prophylactic" or "preventing" disease conditions. Accordingly, no new matter has been introduced by these amendments.

Rejection: § 112, first paragraph

Claims 1, 3, 4, 7-9, 11, 12, 14-20, 22, 24, 26 and 27 have been rejected under 35 U.S.C. § 112, first paragraph, for failing to comply with the written description requirement. Although applicants disagree with the rejection, and disagree that the classification of the compound is relevant to the written restriction requirement, all claims have been amended in a sincere effort to expedite prosecution of the application. The amendments contained in this reply address each of the items identified in the rejection as raising an issue of failing to comply with the written description requirement. Accordingly, this rejection should be withdrawn.

Claims 1, 3, 4, 7-9, 11, 12, 14-20, 22, 24, 26 and 27 have been rejected as not being supported by an enabling disclosure. Although applicants disagree with this rejection, in the interest of expediting prosecution of this application, the claims have been amended to coincide with the examiner's determination of that part of the disclosure relative to the variables Y and Ya that is considered enabling. Applicants appreciate the examiner's identification of the subject matter that was regarded as enabling - very helpful in this case. Accordingly, this rejection should be withdrawn.

Claims 22, 24 and 26 have been rejected under 35 U.S.C. § 112, first paragraph, as not being supported by an enabling disclosure for the treatment of diabetes type II or obesity, and for the prevention of diabetes type 1 or 2 as well as obesity. Although applicants do not agree with this rejection, and again in the interest of expediting the prosecution of this application, these claims have been amended to delete reference to diabetes complications and to "preventing" these disease conditions.

It is respectfully submitted that the claims as amended are supported by an enabling disclosure since Boehm and Lustig, "Use of Somatostatin receptor ligands in obesity and diabetic complications," Best Practice & Research Clinical Gastroenterology, Vol. 16, No. 3, pp. 493-509 (2002) (copy attached), describes the correlation between inhibition of somatostatin (SMS) receptor binding and treatment of obesity and diabetic complications. SMS inhibits the release of growth hormone. *Boehm and Lustig* at 494. "Both obesity and diabetes mellitus are characterized by imbalances of multihormonal systems," including growth hormone and IGF-I. *Id.* SMS and SMS analogs suppress the secretion of growth hormone and reduce the level of IGF-I. *Id.* at 493. SMS and SMS analogs "inhibit insulin secretion." *Id.* at 494.

Accordingly, SMS and SMS analogs serve to lower the levels of growth hormone, IGF-I, and/or insulin.

As each of obesity and diabetes are characterized by common hormonal imbalances, one would expect that ameliorating one or more of these imbalances would provide a benefit in treating either or both of these diseases/conditions. Applicants' claimed compounds inhibit the binding of SMS to its receptor. *Specification* at 1. By inhibiting this binding interaction, endogenous SMS is prevented from decreasing the levels of growth hormone, IGF-I, and/or insulin. Thus, preventing SMS binding is a strategy for increasing the levels of these hormones. The ability to increase the levels of these hormones affords benefits to treating either or both of diabetes and/or obesity because these diseases/conditions share common causes, as discussed above.

Accordingly, this rejection should be withdrawn.

Rejection: § 112, 2d paragraph

Claims 1, 3, 4, 7-9, 11, 12, 14-20, 22, 24, 26 and 27 have been rejected under 35 U.S.C. § 112, second paragraph as being indefinite regarding the substituents for ring A, the variables R^1 and R^2 and the recitation of diabetic complications. While applicants disagree with this rejection since the terms used in these claims would have been understood by those skilled in the art and were not insolubly ambiguous, the amendments made to these claims in a sincere effort to expedite prosecution of this application are considered to avoid these rejections. Accordingly, this rejection should be withdrawn.

Prompt and favorable reconsideration of this application is respectfully requested, and timely issuance of a notice of allowance.

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

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Attachment: Boehm and Lustig, "Use of Somatostatin Receptor Ligands in Obesity and Diabetic Complications," Best Practice & Research Clinical Gastroenterology, Vol. 16, No. 3, pp. 493-509 (2002)

Use of somatostatin receptor ligands in obesity and diabetic complications

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Somatostatin (SMS) is a potent inhibitory molecule. It inhibits both exocrine and endocrine secretory functions of the pancreas, suppresses growth hormone secretion and reduces the level of insulin-like growth factor-I. Long-acting somatostatin analogues were currently investigated for potential clinical benefits in two settings: (a) control of hyperinsulinaemia in obesity and (b) control of an excess of pro-angiogenic factors in diabetes-associated retinal complications. In two randomized, controlled trials the long-acting somatostatin analogue octreotide retarded progression of the microvascular complications in pre-proliferative and advanced stages of diabetic retinopathy. Inhibition of the early phase of insulin secretion by use of octreotide in patients with hypothalamic obesity resulted in weight loss and improved quality of life. Efficacy of octreotide correlated to residual β -cell activity prior to the treatment. Obesity and diabetes mellitus are the most common chronic metabolic disorders in the world. The use of somatostatin analogues addressing the various hormonal imbalances of these disorders may provide a novel concept for their pharmacological treatment.

Key words: diabetic retinopathy; hyperinsulinaemia; hypothalamic obesity; IGF-system; obesity; somatostatin analogues; somatostatin receptors.

Obesity and diabetes mellitus are the most common chronic metabolic disorders in the world.¹ Both diseases can be found in almost all populations. Obesity is a chronic metabolic disorder characterized by an excess of body fat. The classification of obesity is based on the International Obesity Task force recommendation which was made in 1997: pre-obese subjects (body mass index [BMI] 25–29.9), class I obesity (BMI 30–34.9), class II obesity (BMI 35–39.9) and class III obesity as a BMI equal to or greater than 40. The classification defines a strong association of obesity-related co-morbidity. Obesity is associated with increased mortality and morbidity due to hypertension, type 2 diabetes, coronary artery disease, stroke, gallbladder diseases, musculoskeletal problems, including osteoarthritis, sleep apnoea, and other respiratory problems. Various hormonal complications have been described, including menstrual irregularities, hirsutism associated with hyperandrogenaemia, and complications of pregnancy. Also,

prevalence of some cancer types (colon, prostate, breast, endometrium) is associated with an excess of body fat.

There is a clear-cut association of an altered glucose metabolism with obesity, duration of obesity and weight gain. Changes in insulin secretion resulting in hyperinsulinaemia and insulin resistance of peripheral tissues provide important clues in the development of diabetes mellitus. However, diabetes mellitus itself is a heterogeneous group of disorders characterized by a chronic elevated blood glucose level and the propensity to develop specific micro- and macrovascular complications. Typical microvascular complications are diabetic nephropathy and diabetic retinopathy. Macrovascular complications lead to an increased risk for myocardial infarction, stroke gallbladder disease, osteoarthritis, sleep apnoea and limb amputations. Type 2 diabetes is the most common form of diabetes in adults, although the incidence of type 2 diabetes in childhood has risen dramatically by more than 300% in the past 5 years.²

Both obesity and diabetes mellitus are characterized by imbalances of multihormonal systems. Obesity is a phenotype of many diseases; however, hyperinsulinaemia, is a hallmark of obesity. By manipulating this hormonal dysbalance, obesity may become a potentially treatable disease. Both the naturally occurring molecule somatostatin and SMS analogues inhibit insulin secretion.^{3,4} Therefore these molecules may be attractive drugs in the treatment of hyperinsulinaemia.

As in obesity, diabetes mellitus is also characterized by multihormonal imbalances, one of which refers to growth hormone (GH) and the IGF-I system.⁵⁻⁷ Because growth hormone and IGF-I system components act as permissive factors of pro-angiogenic signals, their modulation and/or inhibition by use of the inhibitory substance peptide somatostatin, or by SMS analogues, is also an attractive target for the treatment of vascular complications of diabetes mellitus with neoangiogenesis.

SOMATOSTATIN (SMS)

The peptide somatostatin (SMS) was defined in the 1960s as a molecule that inhibits the release of growth hormone. The molecule was found almost accidentally during studies of the distribution of growth-hormone-releasing factor in the hypothalamus of rats.^{8,9}

SMS is synthesized as a precursor molecule. After enzymatic cleavage, two active peptides, somatostatin-28 and somatostatin-14, are generated. These molecules are ligands for a specific set of somatostatin-binding receptors (sstrs).^{10,11}

Somatostatin can be found throughout the brain and spinal cord and also in the intestine. Like somatostatin, its receptors have been found to be located ubiquitously throughout the body.¹² However, the various subtypes of receptor are differentially expressed at the target sites of SMS action. Five subtypes of receptor have been described thus far, each of which is encoded by one of five independent genes located on different chromosomes. The biological effects are mediated by these five membrane-bound specific receptors, called sstr 1-5 (Tables 1 and 2). All receptors are G protein-coupled receptors with seven transmembrane spanning domains linked to adenylate cyclase.^{13,14}

The physiological actions of somatostatin are primarily inhibitory^{13,14} (Tables 1, 2). SMS affects, for example, calcium and potassium ion channels, leads to tyrosine phosphatase activation and may therefore modulate transmission of neuroendocrine cells, modulate their secretion, and may also affect cell proliferation at several organ systems. Effects have recently been found on the retina and vascular endothelial

Table 1. Actions of somatostatin.

Organ	Action
Pituitary	GH ↓, TSH ↓
Endocrine pancreas	Insulin ↓, Glucagon ↓, Pancreatic Polypeptide ↓
Exocrine pancreas	Bicarbonate secretion ↓, Enzyme secretion ↓
Thyroid	T4 ↓, T3 ↓, Calcitonin secretion ↓
Gastrointestinal tract	Mesenteric blood flow ↓ Mucosal cell proliferation ↓ Gastrin ↓, Secretin ↓, CCK ↓, VIP ↓, GIP ↓ Motilin ↓, Enteroglucagon ↓, Neurotensin ↓ Gastric acid ↓, Pepsin ↓, Intrinsic factor ↓ Gastric emptying ↓ Gallbladder contraction ↓ Small intestine segmentation ↓ Colonic fluid secretion ↓ Bile secretion ↓

cells.^{6,15} SMS acts as a classical hormone, a neurohormone, as a neurotransmitter and exerts autocrine and paracrine functions.

SMS analogues

The naturally occurring somatostatin has a very short half-life of only 1.5–3 minutes; this pharmacological peculiarity requires a continuous intravenous route for the application of SMS. A post-infusion hypersecretion of growth hormone is also found. To address these limitations synthetic analogues of the molecule have been developed.⁴ Each of the synthetic analogues incorporates four amino acids in a specific configuration. The various somatostatin receptor agonists bind to each of the ssr subtypes with varied affinities (Table 3). Only a very recently described analogue

Table 2. Somatostatin receptor subtype expression.

Subtype	1	2	3	4	5
Chromosomal location	14	17	22	20	16
Distribution					
1. Brain	+	+	+	+	+
2. Eye	—	+	—	—	—
3. Kidneys	+	—	—	—	—
4. Exocrine Pancreas	+	—	+	—	—
Endocrine Pancreas	—	+	—	—	+
5. Pituitary	—	—	—	—	+
6. Heart	—	—	—	—	+
G protein coupling	+	+	+	+	+
K ⁺ /Ca ²⁺ channels	—	+	—	—	—
Adenyl cyclase coupling	+	+	+	+	+

(SOM230) was found to bind almost like the naturally occurring SMS peptides to all strrs with equal affinity to exert equal potency.¹⁶ Non-peptidergic receptor analogues may provide a non-parenteral usage of SMS analogues in clinical medicine.¹⁷

DIABETES MELLITUS

Progressive damage to the eyes, kidneys, nerves and large vessels represents the major threat to health and life of diabetic patients.^{1,18} Therefore, prevention of complications and disability due to the chronic hyperglycaemic state should be undertaken in order to alleviate the burden of diabetes-specific complications.¹

Diabetic retinopathy

Retinopathy is the most frequent and specific chronic microvascular complication of diabetes mellitus. It represents a major threat to eyesight in the Western countries, and is the leading cause of blindness among people of working age.^{19,20} Although strict glucose control can delay the onset of diabetic retinopathy and ameliorate the severity of the disease, both in type 1 and type 2 diabetes, epidemiological data show that the incidence of diabetic retinopathy remains strikingly high.¹⁹⁻²¹

Diabetic retinopathy (DR) can be classified into three stages: non-proliferative retinopathy, which does not impair vision, proliferative retinopathy, which impairs the vision, and ongoing proliferations of the vasculature which may lead to vitreo-retinal surgical approaches to preserve some vision. Loss of vision due to diabetic retinopathy involves several mechanisms. Central vision may be impaired by either macular oedema, or ischaemic maculopathy. New pathological blood vessels, which appear at the proliferative phase of the disease, lead to visual impairment by vitreous haemorrhage. The development of proliferative vitreoretinopathy accompanied by contraction of the fibrous tissue can distort the retina, thus leading to tractional retinal detachment.

After 20 years, diabetic retinopathy can be detected in almost all patients with type 1 diabetes mellitus (80–95%) and in a majority of type 2 diabetic patients (50–80%).

Glucose hypothesis of diabetic retinopathy and its prevention

Two large randomized prospective clinical trials showed the important role of glucose levels in the development of diabetic complications ('glucose hypothesis'). Intensive conventional insulin therapy (ICT) in type 1 diabetic patients reduced or prevented the development of retinopathy by 27% as compared with conventional therapy (primary prevention) in the Diabetes Control and Complications Trial (DCCT).²² Glycosylated haemoglobin 1c (HbA1c) levels in the intensified treated group were about 7.0% versus 9.0% in the conventionally treated group; almost half of the patients in the intensified treated group reached an HbA1c level of 6.1% or less at least once during the study period. Thus, intensified insulin regimen significantly diminished the progression of diabetic retinopathy (secondary prevention). Most importantly, early treatment with ICT was most effective.

In the United Kingdom Prospective Diabetes Study (UKPDS) trial an intensified treatment (insulin treatment and/or use of various oral agents) of newly diagnosed type 2 diabetic patients lowered HbA1c to a median level of 7.0% and decreased the probability of microvascular complications by 25%.²³

Table 3. In vitro binding to human recombinant somatostatin receptors.

Subtype	1	2	3	4	5
Peptide					
SRIF-14	2.26	0.23	1.16	1.76	1.41
SRIF-28	2.38	0.29	1.26	2.93	0.40
Lanreotide	2414	0.75	97.8	1826	5.21
Octreotide	876	0.57	26.7	5030	6.77

Ki (nM) are given; a low number reflects high-affinity binding. Data indicate that both SMS analogues bind with high affinity to sstr 2 and sstr 5 receptors.

From both trials it has become clear that lowering glucose levels per se justifies the widespread use of the so-called intensified treatment regimens in diabetics. Lowering of blood glucose levels was found to be effective in both type 1 and type 2 diabetic patients in lowering the likelihood of the presentation and the progression of microvascular complications. However, no evidence for a glucose threshold could be found.²⁴⁻²⁶ If glucose lowering fails, photocoagulation is an important treatment modality. However, after photocoagulation visual acuity may decrease and constriction of peripheral visual field occurs.^{19,20} It is still a matter of debate whether so-called early photocoagulation in patients with mild-to-severe non-proliferative or early proliferative diabetic retinopathy is justified.

Growth hormone and the IGF-I system

Diabetes mellitus is characterized by multihormonal imbalances which include the growth hormone (GH) and IGF-I system. GH has been implicated as an important mediator of diabetic retinopathy. Diabetic patients with a life-long growth hormone deficiency rarely develop retinopathy. In addition, pituitary ablation in patients with retinopathy has been shown to lead to an improvement of this microvascular complication.^{7,19,27}

Alterations in secretion of growth hormone, altered response to the hypothalamic suppressor somatostatin, excess of growth hormone-releasing hormone (GHRH), or an altered responsiveness to insulin-like growth factor I (IGF-I), which also contributes to the negative feedback loop of the GH secretion, are all implicated in the observed changes in growth hormone levels.⁴

The development of DR is a multifactorial process with a disturbed milieu of growth factors and inhibitory molecules which can be produced locally and which can also result from the vasculature due to the breakage of the blood-retina barrier. It was shown recently that the vasculature of the normal retina is maintained by the action of a natural anti-angiogenic factor derived from the pigment epithelium. This factor, which has been named pigment epithelium derived factor (PEDF) controls the vasculature and maintains its quiescent state even when the strong pro-angiogenic factor VEGF is increased.^{28,29} A low level of PEDF was found to strongly predict the development of either pre-proliferative or proliferative retinopathy. A reciprocal fall in inhibitory PEDF was needed before pro-angiogenic factors could act as stimulatory molecules to the vasculature. It appears that PEDF acts as a brake on VEGF and other stimulatory factors and that it is only when this brake fails that pathological retinopathy can develop.^{28,30,31}

In addition, insulin-like growth factor components play an important role as permissive factors for VEGF action, and the inhibition of this molecule was shown to control neo-angiogenesis in an animal model of retinopathy.⁶

Treatment of diabetic retinopathy with somatostatin and somatostatin analogues

Somatostatin or somatostatin analogues can suppress the secretion induced by growth hormone releasing hormone (GHRH).^{3,4} In the 1960s and 1970s – when somatostatin was not yet available – inhibition of GH secretion by hypophysectomy was found to be a clinically effective treatment of DR.³²

Table 4 summarizes the treatment protocols published thus far which have used somatostatin analogues in the context of diabetic retinopathy (summarized in ref. 33; Table 4). Various dosages of the somatostatin analogues (minimal dosage per day 150 µg, maximal dosage per day 500 µg of SMS 201–995; 1500 µg/day of BMI23014) have been used in patients with proliferative retinopathy³³ and cystoid macular oedema.^{33,34} Some studies reported effects on the suppression of growth hormone levels, stabilization of neovascularizations, resorption of haemorrhages and the reduction in the number of microaneurysms. In a case report, effective treatment of a macular oedema was also reported.

Recently, two controlled trials have been reported. Grant and co-workers have studied patients with severe non-proliferative diabetic retinopathy or early non-high-risk proliferative retinopathy.³⁵ At this stage of diabetic retinopathy the likelihood for the need of panretinal photocoagulation is high. The SMS analogue octreotide was titrated in 11 patients to the maximally tolerated dose for a 15-month period. Doses of 200 µg/day up to 5000 µg/day of octreotide were used. Only one of 22 eyes of octreotide-treated patients required panretinal photocoagulation, whereas nine of 24 eyes in the control group had to be laser treated. The incidence of ocular disease progression was only 27% in patients treated with octreotide compared with 42% in patients with conventional management. This study provided evidence that octreotide treatment retarded progression of advanced retinopathy and delayed the time for laser photocoagulation. Boehm et al reported the use of octreotide in a cohort of diabetic patients with a very advanced stage of proliferative diabetic retinopathy, i.e. presence of active proliferations after full scatter laser treatment.³⁶ A dose of 300 µg/day of octreotide was used in nine patients; nine patients with standard diabetes management served as controls. The observation period was the longest ever reported in a trial using a somatostatin analogue for the treatment of diabetic complications. After 3 years of treatment the incidence of vitreous haemorrhages was significantly lower in the octreotide-treated patients. Also, visual acuity was preserved and significantly better over time in the octreotide-treated group. Only in the group of octreotide-treated patients did a regression of proliferations, as defined by stereoscopic photography and by the use of fluorescein angiography, occur.

All trials have carefully monitored the safety of SMS use^{30–32} (Table 4). In the Ulm trial no severe hypoglycaemic events, as defined by help needed from third parties or requirement of hospital admissions, occurred during an observational period of almost 3 years. Two patients complained of abdominal discomfort and increased bowel movements, which could be alleviated by the use of an oral pancreatic enzyme supplementation. No gall bladder stones or sludges were noted on the routine ultrasound examinations of the abdomen.³⁶

Ongoing clinical trials are currently evaluating the possible effect of a sustained release form of octreotide in patients with severe non-proliferative and early proliferative

Table 4. Trials using somatostatin and somatostatin analogues for the treatment of diabetic retinopathy.

Author and citation	Number of patients	Treatment	Duration	Effects	Side-effects
<i>Uncontrolled trials</i>					
Mallet et al (1992)	4 (type 1)	SHS 201-995 continuous s.c. infusion 44 µg/day	6-20 (mean 15) months	Two patients: neovascularization stopped Two patients: neovascularization regressed	Insulin doses decreased, diabetic diarrhoeas, gallbladder stones (1)
Lee et al (1996)	Case report (type 1)	SHS 201-995 3 × 50 µg s.c. per day	3 months	Complete resorption of haemorrhages Reduction in numbers of microaneurysms and areas of extravasillary dye leakage	None
Shumak et al (1990)	6 (type 1)	SHS 201-995 3 × 100 µg per day	8 weeks	Improvement of retinopathy in two patients	None
Kuipers et al (1998)	Case report	SHS 201-995 s.c. 3 × 100 µg per day	12 weeks	Macular oedema dried, visual acuity improved, but rebound effect	
<i>Controlled trials</i>					
Hyer et al (1989)	9 (type 1) (6 controls)	(A) SHS 201-995 3 × 50 µg s.c. per day (B) Continuous s.c. infusion 500 µg/day	(A) 20 weeks (B) 3 days to 16 weeks	(A) No GH suppression, visual acuity minus 2 lines in 3/4 patients (B) Complete/partial suppression of GH level, no change to visual acuity (5 patients), improvement (1 patient). No prevention of retinal haemorrhages and further laser treatment	Abdominal discomfort, trend to hypoglycaemia

Table 4 continued over page

Table 4. Continued

Author and citation	Number of patients	Treatment	Duration	Effects	Side-effects
Kirkegaard et al (1990)	7 (11) (9 controls)	SHS 201-995 continuous s.c. infusion 400 µg/day	12 months	No effects, no difference between the two groups	Decline in insulin requirements, mild transient hypothyroidism
McCombe et al (1991)	11 (6 controls)	BN1123014 continuous s.c. infusion 1800 µg/day	3 months	Two patients: clinical and angiographic improvement; 1 patient: no effect	
Grant et al (2000)	11 (12 controls)	SHS 201-995 s.c. 200-5000 µg/day	15 months	Progression to hypothyroidism; retinopathy was significantly reduced; progression was seen in 27% of SHS treated patients, in 42% of controls	Decline in insulin requirements, replacement therapy with thyroxine
Boehm et al (2001)	9 (9 controls)	SHS 201-995 s.c. 300 µg/day	36 months	Episodes of vitreous haemorrhages and need for vitreoretinal surgery were reduced significantly by use of SHS	Up to 40% decline in insulin requirements in type 1 patients

Miller et al (1992) *Diabetes et Metabolismus* 18: 438; Lee et al (1998) *Diabetes Care* 11: 441; Shumak et al (1990) *Clinical and Investigative Medicine* 13: 287; Kuntjpers et al (1998) *New England Journal of Medicine* 338: 624; Hyer et al (1997) *Acta Endocrinologica* 126: 167; Kirkegaard et al (1990) *Acta Endocrinologica* 122: 766; McCombe et al (1991) *Eur J* 5: 569; Grant et al (2000) *Diabetes Care* 23: 504; Boehm et al (2001) *Hormone and Metabolic Research* 33: 300.

diabetic retinopathy. The effect on macular oedema is also studied because case reports demonstrated that somatostatin analogues inhibit vascular effusions.^{15,34}

Other effects of SMS on eye diseases

The expression pattern of both somatostatin and its receptor subtype 2 on other cellular elements of the human eye makes it highly likely that SMS exerts direct regulatory functions.¹⁵ The observed positive effects in cystic maculopathy and the positive case reports in patients with macular oedema make it likely that SMS controls fluid evasion or resorption.³¹ Most probably the pigment epithelium cell, which can produce pro- and anti-angiogenic molecules, is responsible for a favourable exchange of fluids.^{27,28} SMS was also used in age-related macular degeneration. It was found to act in the so-called presumed ocular histoplasmosis syndrome, thus extending the modulatory effects of SMS in other ocular neovascularization diseases.^{30,36}

The future role of pharmacotherapy of diabetic retinopathy

The long-acting analogue octreotide (SMS 201–995), as well as other SMS analogues, provides an elegant pharmacological principle to modify the high-risk form of proliferative diabetic retinopathy.³⁰ The potential role of these substances may be due to suppression of pro-angiogenic molecules, a direct inhibition of pro-angiogenic signalling at the cell level, an anti-fibrotic action, thus reducing fibrovascular formations, and at least a partial correction of the systemic growth hormone and IGF-I dysregulation.^{5–7,15,30,31,37–39} Data from the Ulm trial and from other pilot investigations have provided evidence that octreotide can very effectively suppress new bleedings and stop visual loss in patients who have failed conventional photocoagulation therapy.^{30,35,37} In this cohort octreotide was found to be a safe treatment modality. It remains to be clarified whether the progression from pre-proliferative to proliferative retinopathy can also be stopped by the use of the long-acting somatostatin analogues.^{15,30,40,41}

OBESITY

The incidence of obesity worldwide is climbing at an alarming rate.⁴² The fallacy of obesity is that it can be attributable to either increased caloric intake or decreased caloric expenditure only. In fact, the aetiologies and pathogeneses of obesity are myriad⁴³, as are the potential therapies⁴⁴, but a rational approach to obesity is dependent on the development of a useful nosology with consistent and accurate diagnostic criteria.

One clear feature shared by all obesity subtypes is an excess of adipose tissue. Excess blood-borne energy substrates (glucose, fatty acids) which are not oxidized immediately by other tissues are stored in adipose via the lipogenic effects of the hormone insulin. The amplitude and duration of pancreatic insulin secretion and the activity of the insulin molecule at the adipose insulin receptor both play important roles in the genesis of lipogenesis and weight gain. This is supported by the fact that hyperinsulinaemia is a component of most animal models of obesity (eg. *ob*, *db*, *fa*, *Ay*).⁴⁵ Insulin is the primary hormonal mediator of adipogenesis in humans.⁴⁶ Within the adipocyte, insulin regulates: (a) Glut4 expression; (b) acetyl-CoA carboxylase; (c) fatty acid synthase; and (d) lipoprotein lipase.⁴⁷ The overwhelming majority of obese humans exhibit hyperinsulinism and insulin resistance as hallmarks of their obesity.^{48,49}

Hyperinsulinism, insulin resistance, and obesity are inexorably linked through reciprocal control mechanisms. Hyperinsulinaemia may cause insulin resistance through changes in glucose transport^{50,51} or through insulin's down-regulation of its own receptor^{52,53}, and can cause lipogenesis and increased weight gain directly, as is noted in infants of diabetic mothers.^{54,55} Conversely, insulin resistance may cause compensatory hyperinsulinaemia through poorly understood reflex mechanisms⁵⁶, and can cause lipogenesis through up-regulation of the Glut4 transporter and the enzymes acetyl-CoA carboxylase and fatty acid synthase.^{47,57} Lastly, the degree of obesity exhibits a direct correlation with the degree of insulin resistance.^{58,59} Although the debate continues on which comes first – the insulin secretion, the insulin resistance, or the obesity^{60,61} – these concepts clearly suggest that insulin is a potential target for obesity pharmacotherapy.⁶² Acute glucose-stimulated insulin hypersecretion in insulin-sensitive adults has been shown to predict future weight gain.⁶³ Similarly, in children, an augmented early post-prandial insulin response precedes the development of obesity.⁶⁴ Such studies lend credence to the hypothesis that insulin hypersecretion is a risk factor for the development of obesity in humans.

Neural regulation of β -cell function

The regulation of β -cell function is extremely complex; in this process neural, endocrine, and paracrine factors all interact to control insulin secretion. The ventromedial hypothalamus (VMH) coordinates energy balance through both sympathetic and parasympathetic inputs to the β -cell. Damage to the VMH in rats leads to hyperphagia, hyperinsulinaemia and obesity.^{65–68} There is ample evidence that abnormally increased vagal tone with resultant insulin hypersecretion mediate this phenomenon.^{69–78} The vagus may also promote insulin hypersecretion through stimulation of intestinal L-cells to produce the incretin glucagon-like peptide-I (GLP-1), which reaches the β -cell haematogenously and binds to specific receptors to augment the insulin response to glucose.^{79,80}

Somatostatinergic regulation of β -cell function

Rodent β -cells possess sstr-5 receptors, as determined by immunocytochemical⁸¹, molecular biological⁸² and combinatorial chemical¹⁷ methods; however, in humans there is still some debate as to whether the subtype sstr 2 predominates. In both species, the sstr is coupled to the voltage-gated calcium channel, and SMS exerts an inhibitory action on channel opening.⁸³ In the presence of SMS or analogue, the magnitude of the calcium influx is attenuated, which limits the amplitude of the early insulin response to glucose. Acutely, SMS suppression of insulin secretion may cause hyperglycaemia⁸⁴, but this is rapidly normalized within a few days, while the suppression of the early insulin response to glucose limits the compartmentalization of energy substrate into adipose.

The use of somatostatin analogues in obesity

SMS and its analogues have ubiquitous physiological actions which can be exploited through selective binding to different sstr subtypes.^{13–16} Nowhere is this better exemplified than in the use of the SMS analogue octreotide for the treatment of a subtype of obesity due to vagal dysregulation of pancreatic β -cell function and insulin excess.

Octreotide therapy of hypothalamic obesity

Experimental bilateral electrolytic lesions or de-afferentation of the VMH in rats leads to hyperphagia and intractable weight gain, termed 'hypothalamic obesity'.^{71,85,86} Similarly in humans, hypothalamic damage from a tumour, surgery or radiation often results in unrelenting weight gain, which is not amenable to caloric restriction or exercise.^{66,67-90} Hypothalamic obesity is probably due to VMH neuronal damage with an alteration in leptin feedback.⁶⁷ Indeed, caloric restriction of either VMH-lesioned animals or patients with hypothalamic obesity does not attenuate the weight gain⁹¹, suggesting that energy expenditure is also affected. Patients with hypothalamic obesity manifest an increase in vagal firing rate which promotes insulin hypersecretion.^{72,73} The excess insulin causes shunting of blood-borne energy substrate to adipose.⁹⁰ Furthermore, leptin, insulin and ghrelin resistance due to hypothalamic damage would prevent any afferent feedback effects on caloric intake, leading to continued weight gain.⁶⁷ This would result in an obesity syndrome with high leptin, low fasting insulin, and high and early peak insulin on OGTT.

We analysed the insulin excursion to oral glucose tolerance testing (OGTT) in eight children with intractable obesity secondary to tumour therapy.⁹² Our subjects exhibited only slightly elevated fasting insulin concentrations. However, they demonstrated accentuated early insulin responses to OGTT, which were elevated in comparison to their degree of obesity. Peak insulin levels were reached by 60 minutes, and were $281 \pm 47 \mu\text{U/ml}$, as compared to $8-150 \mu\text{U/ml}$ in normal adolescents. The fall to baseline was rapid, suggesting that these patients did not exhibit defective insulin clearance, as is usually seen with obesity due to insulin resistance.⁵⁹

The early phase of insulin secretion can be pharmacologically inhibited by limiting the opening of the voltage-gated calcium channel using the SMS analogue octreotide.⁴ In an open-labelled pilot trial⁹³, eight patients with hypothalamic obesity received subcutaneously administered octreotide for 6 months, at a dose of $5 \mu\text{g/kg/day}$ tid, escalating in monthly $5 \mu\text{g/kg/day}$ increments to a maximum of $15 \mu\text{g/kg/day}$ tid. Insulin responses to glucose were normalized over 6 months. Of the eight patients, three lost substantial weight, two lost moderate amounts of weight, and three stabilized their weight. The degree of weight loss correlated both with changes in insulin response and changes in leptin levels. The weight loss also appeared to correlate with decreases in appetite as caloric intake in this cohort decreased by approximately 700 kcal/day. The lack of leptin negative feedback by the damaged VMH was not critical as these patients responded with weight loss, decreased caloric intake and decreased leptin levels. In fact, an unexpected but welcome side-effect of the treatment was the resumption of normal physical activity by four patients, including vigorous exercise.

A double-blind, placebo-controlled 6-month trial of octreotide in 18 subjects with hypothalamic obesity has now been completed.⁹³ Prior to treatment, annualized weight gain of these patients was $15.9 \pm 2.9 \text{ kg/year}$. Although the weight loss in this trial was not as pronounced, octreotide was effective in stabilizing weight ($+1.6 \pm 0.6 \text{ kg}$) and BMI ($-0.1 \pm 0.1 \text{ kg/m}^2$), as compared to placebo (change in weight $+9.2 \pm 1.5 \text{ kg}$, BMI $+2.3 \pm 0.5 \text{ kg/m}^2$). Insulin secretion was clearly suppressed by octreotide therapy. Lastly, a measurement of quality of life demonstrated marked improvements in physical activity as compared with those treated with placebo. These data suggest that insulin hypersecretion is responsible not only for weight gain in these patients, but for their lack of physical activity, which can be improved by normalizing their insulin response. Anecdotally, patients noted normal hunger at the time of meals but noted the lack of drive to continue to eat past their

first serving, or for snacking between meals. They also noted more interest in their schoolwork and in their social contacts. Treatment of those initially assigned to placebo resulted in stabilization of weight and improved quality of life as well.

Octreotide therapy of adult obesity due to primary insulin hypersecretion

We hoped to identify a subpopulation of obese adults who also demonstrated insulin hypersecretion⁶³ but without obvious cranial pathology. Forty-four severely obese (BMI > 35 kg/m²) but otherwise healthy adults were treated in an open-label fashion with octreotide-LAR, 40 mg intramuscularly every 28 days for a 6-month period, without changes in lifestyle, diet or exercise.⁹⁴ Of the 44 subjects, eight exhibited substantial weight loss (mean 13 kg). The eight responders exhibited significantly different baseline insulin dynamics to OGTT compared to the other 36 subjects, with a rapid insulin rise peaking at 60 minutes, and an equally rapid fall. Octreotide suppressed this insulin rise, without glucose intolerance. Again, the change in leptin correlated with the change in BMI, suggesting reduction in adipose stores. The degree of pre-treatment β -cell activity was an a priori predictor of response to octreotide: the higher the pre-treatment β -cell activity the more weight was lost. Furthermore, vagal modulation correlated directly with β -cell activity in these obese patients.⁹⁵

Of course, this does not preclude other possible mechanisms of octreotide action to reduce weight and BMI. Modulation of other GI hormones, such as glucagon-like peptide-1^{79,80}; slowing of gastric emptying and GI motility, with nutrient malabsorption⁹⁶; direct effects on appetite^{97,98}, or direct effects on the adipocyte^{99,100} have all been reported with SMS or its analogues. However, these other mechanisms seem less likely, as GI symptoms and appetite suppression were uniformly distributed throughout all response strata (data not shown), and only those subjects who exhibited weight loss demonstrated insulin suppression, as exhibited by decreases in C-peptide and insulin excursions, and decreases in IAUC.¹⁰¹ Furthermore, if another mechanism, other than insulin suppression, was responsible for the weight loss, subjects receiving octreotide for acromegaly or other disorders would be expected to lose weight and fat mass; indeed, long-term octreotide usage has minimal effects on these parameters.¹⁰² Lastly, SMS has been shown to reduce food intake in animals only

Table 5. Applications of somatostatin analogues.

<i>Established applications</i>
Pituitary adenomas: acromegaly, TSHoma
Metastatic islet-cell tumours
Metastatic neuroendocrine tumours
Somatostatin-receptor scintigraphy
Chemotherapy-induced diarrhoea
Acute oesophageal varicea bleeding
<i>Probable applications</i>
Pancreatic and enteric fistulas
Secretory diarrhoea
AIDS-related diarrhoea
<i>Possible applications, clinical applications under investigation</i>
Diabetic microvascular complications: retinopathy, nephropathy
Obesity due to insulin hypersecretion
Graves ophthalmopathy
Radiotherapy of somatostatin-receptor-positive diseases

if their vagus nerve is left intact.⁹⁸ This suggests that the decline in caloric intake with SMS or its analogues is mediated through suppression of β -cell depolarization.

SUMMARY

Obesity and diabetes mellitus are the most common chronic metabolic disorders in the world. A massive increase of insulin secretion is typical of obesity, whereas an increase of growth hormone secretion and changes in components of the IGF-I system are paradigmatic of the dysglycaemic syndrome. Because the actions of somatostatin and somatostatin analogues are primarily inhibitory, these molecules may provide novel treatment modalities. However, before the widespread use of these inhibitory molecules can occur, our understanding of the pathophysiology of obesity and diabetes mellitus has to be changed. For example, diabetes mellitus not only requires the substitution of insulin for a balanced glucose level but also a direct modification of the imbalance of the IGF-I system. From a historical standpoint these are new implications of concepts almost 30 years old.^{103,104} The relative merits of SMS treatment of obesity and diabetic complications remain to be assessed in large multicentre investigations. Nevertheless, currently available SMS analogues have already allowed the modulation of the expression of obesity and the expression of diabetic retinopathy at various stages of disease and, as such, provide a novel concept in endocrinological practice (Table 5).

Practice points

- obesity and diabetes mellitus are characterized by imbalances of multihormonal systems, including insulin, and growth hormone and IGF-I system components
- long-term somatostatin analogue treatment is safe and can retard progression of retinopathy and preserve vision
- long-term somatostatin analogue treatment can retard weight gain and suppress hyperinsulinaemia in obesity

Research agenda

- clinical research will need to demonstrate the efficacy of somatostatin analogue treatment for prevention and retardation of diabetic retinopathy
- clinical research will need to demonstrate the efficacy of somatostatin analogues in the treatment of obesity, including the demonstration of safety in long-term trials

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